



SEQUENCE LISTING

<110> Commonwealth Scientific and Industrial Research Organization

<120> Efficient gene silencing in plants using short dsRNA sequences

<130> BCS-03-2001

<150> US 60/447,711

<151> 2003-02-19

<160> 33

<170> PatentIn version 3.3

<210> 1

<211> 341

<212> DNA

<213> Artificial Sequence

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<221> misc_feature

<222> (1)..(6)

<223> XhoI restriction site

<220>

<221> misc_feature

<222> (7)..(322)

<223> PolIII promoter region

<220>

<221> misc_feature

<222> (323)..(328)

<223> SalI restriction site

<220>

<221> misc_feature

<222> (329)..(335)

<223> poly T nucleotide stretch

<220>

<221> misc_feature

<222> (336)..(341)

<223> XhoI restriction site

<400> 1

ctcgagatgt tgggttacc agaaagtaaa taaatgttca atctctgatg ttctcaagta 60

agtgagtttt attgggaata atattaactc atgttcttct gcatttgatt cctttgccgc 120

tctcttcttc tatcttaat ctgtgtatac tatttcacta ttgggctttt tattagtcta 180

taatgggact caaaataagg ctttggccca cataaaaaag ataagtacaca aatcaaaact 240

aaattcagag tctttctcc cacatcggtc actgtactca ttttgttgc gtttatatat 300

tacacgaacc gatcttggtt acgtcgactt ttttctcga g 341

<210> 2

<211> 429

<212> DNA

<213> Artificial Sequence

<220>
 <223> sequence of the promoter of the 7SL-2 gene of Arabidopsis
 thaliana var. Landsberg erecta including 86 bases downstream of
 the transcription initiation site.

<220>
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 <222> (1)..(6)
 <223> XhoI restriction site

<220>
 <221> misc_feature
 <222> (7)..(408)
 <223> PolIII promoter region.

<220>
 <221> misc_feature
 <222> (409)..(414)
 <223> SalI restriction site

<220>
 <221> misc_feature
 <222> (415)..(423)
 <223> poly T stretch

<220>
 <221> misc_feature
 <222> (424)..(429)
 <223> XhoI restriction site

<400>	2					
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agtgagtttt	attgggaata	atattaactt	atgttcttct	tgcatttgat	ttctttgccg	120
ctctcttctt	cstatctaaa	tctgtgtata	ctatttcact	attgggcctt	ttatttagtct	180
ataatggac	tcaaaataag	gctttggccc	acatcaaaaa	gataagtac	aaatcaaaac	240
taaattcaga	gtctttctc	ccacatcggt	cactgtactc	ttttgtgttt	gtttatatat	300
tacacgaacc	gatcttttgtt	acgtcgagct	aagtaacatg	agcttgtaac	ccatgtgggg	360
acattaagat	ggtggAACAC	tggctcggtt	ccacgggccc	gttctgttgt	cgactttttt	420
tttctcgag						429

<210> 3
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 <213> Artificial Sequence

<220>
 <223> sequence of the promoter of the U3 snRNA of Arabidopsis thaliana
 var. Landsberg erecta

<220>
 <221> misc_feature
 <222> (1)..(6)
 <223> EcoRI restriction site

<220>
 <221> misc_feature
 <222> (7)..(313)

<223> Pol III promoter region
 <220>
 <221> misc_feature
 <222> (314)..(319)
 <223> PvuI restriction site
 <220>
 <221> misc_feature
 <222> (320)..(328)
 <223> poly T stretch
 <220>
 <221> misc_feature
 <222> (329)..(334)
 <223> EcoRI restriction site
 <400> 3
 gaattcttat gcagcctgtg atggataact gaatcaaaca aatggcgctc gggtttaaga 60
 agatctgttt tggctatgtt ggacgaaaca agtgaacttt taggatcaac ttcagtttat 120
 atatggagct tatatcgagc aataagataa gtgggcttt tatgttaattt aatgggctat 180
 cgtccataga ttcactaata cccatgccca gtacccatgt atgcgttca tataagctcc 240
 taatttctcc cacatcgctc aaatctaacc aaatcttggtt gtatatataa cactgaggga 300
 gcaacattgg tcacgatcgt ttttttttga attc 334
 <210> 4
 <211> 467
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> sequence of the promoter of the U3 snRNA gene of *Arabidopsis thaliana* var. Landsberg erecta including 136 bases downstream of the transcription initiation site.
 <220>
 <221> misc_feature
 <222> (1)..(6)
 <223> EcoRI restriction site
 <220>
 <221> misc_feature
 <222> (7)..(446)
 <223> Pol III promoter region
 <220>
 <221> misc_feature
 <222> (447)..(452)
 <223> XbaI restriction site
 <220>
 <221> misc_feature
 <222> (453)..(461)
 <223> poly T stretch
 <220>
 <221> misc_feature
 <222> (462)..(467)
 <223> EcoRI restriction site
 <400> 4

gaattcttat	gcagcctgtg	atggataact	aatcaaaca	aatggcgct	t gggtttaaga	60
agatctgttt	tggctatgtt	ggacgaaaca	agtgaactt	taggatcaac	ttcagtttat	120
atatggagct	tatatcgagc	aataagataa	gtgggctttt	tatgtatatt	aatgggctat	180
cgtccataga	ttcactaata	cccatgccca	gtaccatgt	atgcgttca	tataagctcc	240
taatttctcc	cacatcgctc	aaatctaaac	aaatcttgtt	gtatataaa	caactgaggga	300
gcaacattgg	tcacgacctt	acttgaacag	gatctgttct	ataggctcgt	acctctgttt	360
ccttgatttc	tcaagagaca	ggcccttaac	cctggttgat	gaaccatgac	cgtgcggcta	420
gagcgtgatt	gacggctacg	atcgtcctcg	agttttttt	tgaattc		467

<210> 5
<211> 456
<212> DNA
<213> Artificial Sequence

<220>
<223> sequence of the promoter of the U6-26 snRNA gene of Arabidopsis thaliana var. Landsberg erecta including 3 bases downstream of the transcription initiation site, followed by a unique restriction site in front of an oligo dT stretch

<220>
<221> misc_feature
<222> (1)..(6)
<223> XhoI restriction site

<220>
<221> misc_feature
<222> (7)..(436)
<223> Pol III promoter region

<220>
<221> misc_feature
<222> (437)..(442)
<223> SalI restriction site

<220>
<221> misc_feature
<222> (443)..(450)
<223> poly T stretch

<220>
<221> misc_feature
<222> (451)..(456)
<223> Sac I restriction site

<400> 5	ctcgagcttc	gttgaacaac	ggaaaactcga	cttgccttcc	gcacaataca	tcatttcttc	60
	tttagctttt	ttcttcttct	tcgttcatac	agttttttt	tgttatcag	cttacatttt	120
	cttgaaccgt	agcttcgtt	ttcttctttt	taactttcca	ttcggagttt	ttgtatcttg	180
	tttcatagtt	tgtcccagga	ttagaatgat	taggcatacga	acttcaaga	atttgattga	240
	ataaaacatc	ttcattctta	agatatgaag	ataatcttca	aaaggcccc	ggaaatctga	300
	aagaagagaa	gcaggccat	ttatatggga	aagaacaata	gtatttctta	tataggcccc	360
	tttaagttga	aaacaatctt	caaaagtccc	acatcgctta	gataagaaaa	cgaagctgag	420

tttatataca gctagagtgc actttttt gagctc 456

<210> 6
<211> 488
<212> DNA
<213> Artificial sequence

<220>
<223> sequence of the promoter of the U6-26 snRNA gene of Arabidopsis thaliana var. Landsberg erecta including 20 bases downstream of the transcription initiation site, followed by a unique restriction site in front of an oligo dT stretch

<220>
<221> misc_feature
<222> (1)..(6)
<223> XhoI restriction site

<220>
<221> misc_feature
<222> (7)..(468)
<223> Pol III promoter region

<220>
<221> misc_feature
<222> (469)..(474)
<223> PvuI restriction site

<220>
<221> misc_feature
<222> (475)..(482)
<223> Poly T stretch

<220>
<221> misc_feature
<222> (483)..(488)
<223> XhoI restriction site

<400> 6
ctcgagcttc gttgaacaac ggaaactcga cttgccttcc gcacaataca tcatttcttc 60
tttagctttt ttcttcttct tcgttcatac agttttttt tgtttatcag cttacatttt 120
cttgaaccgt agcttcgtt ttcttcttt taactttcca ttcggagttt ttgtatcttg 180
tttcatagtt tgtcccagga ttagaatgat taggcatacga accttcaaga atttgattga 240
ataaaacatc ttcattctta agatatgaag ataatcttca aaaggccccct gggaatctga 300
aagaagagaa gcaggcccat ttatatggaa aagaacaata gtatttctta tataggccca 360
tttaagttga aaacaatctt caaaaagtccc acatcgctta gataagaaaa cgaagctgag 420
tttatataca gctagagtgc aagtagtgat tgtcccttcg gggacatccg atcgaaaa 480
ttctcgag 488

<210> 7
<211> 405
<212> DNA
<213> Artificial sequence

<220>
<223> sequence of the promoter of the U3 snRNA of rice (*Oryza sativa*)

Indica IR36)

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<220>
<221> misc_feature
<222> (1)..(6)
<223> EcoRI restriction site

<220>
<221> misc_feature
<222> (7)..(384)
<223> Pol III promoter region

<220>
<221> misc_feature
<222> (385)..(390)
<223> PvUI restriction site

<220>
<221> misc_feature
<222> (391)..(399)
<223> poly T stretch

<220>
<221> misc_feature
<222> (400)..(405)
<223> EcoRI restriction site

<400> 7
gaattcaagg gatcttaaa catacgaaca gatcaactaa agttcttctg aagcaactta      60
aagttatcag gcatgcattgg atcttgagg aatcagatgt gcagtcaggg accatagcac
aggacaggcg tcttctactg gtgctaccag caaatgctgg aagccggaa cactgggtac      180
gttggaaacc acgtgatgtg gagtaagata aactgttagga gaaaagcatt tcgttagtggg      240
ccatgaagcc tttcaggaca tgtattgcag tatgggccgg cccattacgc aattggacga      300
caacaaagac tagtattagt accacctcg ctatccacat agatcaaagc tggtttaaaa      360
gagttgtgca gatgatccgt ggcacgatcg tttttttttt aattc      405

<210> 8
<211> 442
<212> DNA
<213> Artificial Sequence

<220>
<223> sequence of the promoter of the U3 snRNA of tomato (a garden
variety with small gourd-shaped yellow fruit)

<220>
<221> misc_feature
<222> (1)..(6)
<223> EcoRI restriction site

<220>
<221> misc_feature
<222> (7)..(421)
<223> Pol III promoter region

<220>
<221> misc_feature
<222> (422)..(427)
<223> PvUI restriction site
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<220>
<221> misc_feature
<222> (428)..(436)
<223> Poly T stretch

<220>
<221> misc_feature
<222> (437)..(442)
<223> EcoRI restriction site

<400> 8
gaattctgag agcattgtgt ggcgttcctc tgaattactt actgtcactt tgattggagc      60
cattattttc agactctact gaagattgaa ttgaatgaga aactatgaaa ctttacaagt      120
gaattattat ggagttcatg gcaactgcta tggagtttt cctactggga attggaacgg      180
tttctacgaa attaactgtc cacacgttaa aaatataaat taatgcgtaa ttgttatttt      240
ttctataaca aataaaaaac tgaaatacga cataaatttt attactttaa ttgcacttta      300
gccttagaga tattgcgttg tagtcggcgt aggtgtgtca ggggccaata tattgttccc      360
acatcggcag tgcagcacat aaactctagc gttataagaa tctatccact atcaacggtc      420
acgatcgaaa ttttttgaat tc                                         442

<210> 9
<211> 295
<212> DNA
<213> Artificial Sequence

<220>
<223> sequence of the dsRNA encoding region of 94bp for silencing
      expression of the GUS gene (GUShp94)

<220>
<221> misc_feature
<222> (1)..(6)
<223> SalI restriction site

<220>
<221> misc_feature
<222> (6)..(11)
<223> PvUI restriction site

<220>
<221> misc_feature
<222> (12)..(100)
<223> GUS sequence (sense)

<220>
<221> misc_feature
<222> (101)..(195)
<223> spacer sequence

<220>
<221> misc_feature
<222> (190)..(195)
<223> BamHI restriction site

<220>
<221> misc_feature
<222> (196)..(284)
<223> GUS sequence (antisense)

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<220>
<221> misc_feature
<222> (285)..(290)
<223> PvuI restriction site

<220>
<221> misc_feature
<222> (290)..(295)
<223> SalI restriction site

<400> 9
gtcgacgatc gcagcgtaat gctctacacc acgccgaaca cctgggtgga cgatatcacc      60
gtggtgacgc atgtcgcgca agactgtAAC cacgcgtctg ttgactggca ggtggtggcc      120
aatggtgatg tcagcgTTGA actgcgtgat gcggatcaac aggtggttgc aactggacaa      180
ggcactagcg ggatccagac gcgtggttac agtcttgcgc gacatgcgtc accacggtga      240
tatcgTccac ccaggtgttc ggcgtggtgt agagcattac gctgcgatcg tcgac      295

<210> 10
<211> 93
<212> DNA
<213> Artificial Sequence

<220>
<223> sequence of the dsRNA encoding region of 41 bp for silencing
expression of the GUS gene (GUShp41)

<220>
<221> misc_feature
<222> (1)..(6)
<223> SalI restriction site

<220>
<221> misc_feature
<222> (7)..(42)
<223> GUS sequence (sense)

<220>
<221> misc_feature
<222> (43)..(51)
<223> spacer sequence

<220>
<221> misc_feature
<222> (52)..(87)
<223> GUS sequence (antisense)

<220>
<221> misc_feature
<222> (88)..(93)
<223> Sal I restriction site

<400> 10
gtcgactggg cagatgaaca tggcatcgTG gtgattgatg aatgcgagaa ctTCatcaat      60
caccacgatg ccatgttcat ctgcccagTC gac      93

<210> 11
<211> 50
<212> DNA
<213> Artificial Sequence

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<220>
<223> sequence of the dsRNA encoding region of 21 bp for silencing
      expression of the GUS gene (GUShp21)

<220>
<221> misc_feature
<222> (1)..(6)
<223> SalI restriction site

<220>
<221> misc_feature
<222> (7)..(22)
<223> GUS sequence (sense)

<220>
<221> misc_feature
<222> (23)..(28)
<223> spacer sequence

<220>
<221> misc_feature
<222> (29)..(44)
<223> GUS sequence (antisense)

<220>
<221> misc_feature
<222> (45)..(50)
<223> Sal I restriction site

<400> 11
gtcgactggg cagatgaaca tgtacgatca tgttcatctg cccagtcgac      50

<210> 12
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> sequence of the dsRNA encoding region of 42 bp for silencing
      expression of the PHYB gene, derived from the 5' end of PHYB
      (PHYB5hp42)-upper strand

<400> 12
tcgacggagt cggggtagt ggcggtgccc gtggcggtgg ccgtggagga gcccacggcc      60
accgccacgg ccaccgcccc taccccccac tccg                          94

<210> 13
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> sequence of the dsRNA encoding region of 42 bp for silencing
      expression of the PHYB gene, derived from the 5' end of PHYB
      (PHYB5hp42)-lower strand

<400> 13
tcgacggagt cggggtagt ggcggtgccc gtggcggtgg ccgtggcctc ctccacggcc      60
accgccacgg ccaccgcccc taccccccac tccg                          94

<210> 14

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<211>	52			
<212>	DNA			
<213>	Artificial Sequence			
<220>				
<223>	sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the 5' end of PHYB (PHYB5hp21)-upper strand			
<400>	14			
	tcgacggagt cggggtagt ggcggaggag gccgccacta ccccccgactc cg			52
<210>	15			
<211>	52			
<212>	DNA			
<213>	Artificial Sequence			
<220>				
<223>	sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the 5' end of PHYB (PHYB5hp21)-lower strand			
<400>	15			
	tcgacggagt cggggtagt ggccgcctcc tccgccacta ccccccgactc cg			52
<210>	16			
<211>	94			
<212>	DNA			
<213>	Artificial Sequence			
<220>				
<223>	sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp42)-upper strand			
<400>	16			
	tcgatggatg gtgtggttca gccatgtagg gatatggcg ggaaacagga gggttcccc			60
	gccatatccc tacatggctg aaccacacca tcca			94
<210>	17			
<211>	94			
<212>	DNA			
<213>	Artificial Sequence			
<220>				
<223>	sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp42)-lower strand			
<400>	17			
	tcgatggatg gtgtggttca gccatgtagg gatatggcg ggaaaccctc ctgttcccc			60
	gccatatccc tacatggctg aaccacacca tcca			94
<210>	18			
<211>	52			
<212>	DNA			
<213>	Artificial Sequence			
<220>				
<223>	sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp21)-upper strand			

<400> 18	tcgatggatg gtgtggttca gccataggag gatggctgaa ccacacctcc aa	52
<210> 19		
<211> 52		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp21)-lower strand		
<400> 19	tcgatggatg gtgtggttca gccatcctcc tatggctgaa ccacaccatc ca	52
<210> 20		
<211> 94		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the 3' end of PHYB (PHYB3hp42)-upper strand		
<400> 20	tcgacattgt caactgctag tggaagtgg t gacatgatgc t gatgaagga ggtcatcagc	60
	atcatgtcac cacttccact agcagttgac aatg	94
<210> 21		
<211> 94		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the 3' end of PHYB (PHYB3hp42)-lower strand		
<400> 21	tcgacattgt caactgctag tggaagtgg t gacatgatgc t gatgacctc ctcatcagc	60
	atcatgtcac cacttccact agcagttgac aatg	94
<210> 22		
<211> 52		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the 3' end of PHYB (PHYB3hp21)-upper strand		
<400> 22	tcgacattgt caactgctag tgaaaaggag gttccactag cagttgacaa tg	52
<210> 23		
<211> 52		
<212> DNA		

<213> Artificial Sequence
 <220>
 <223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the 3' end of PHYB (PHYB3hp21)-lower strand
 <400> 23
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<210> 24
 <211> 94
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PDS gene (PDS42)-upper strand
 <400> 24
 tcgacttaac ttgtaggaa tattacgatc ctaaccggtc aatgcttagga ggagcattga 60
 ccggtagga tcgtaatatt ccttacaagt taag 94

<210> 25
 <211> 94
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PDS gene (PDS42)-lower strand
 <400> 25
 tcgacttaac ttgtaggaa tattacgatc ctaaccggtc aatgctcctc ctgcattga 60
 ccggtagga tcgtaatatt ccttacaagt taag 94

<210> 26
 <211> 52
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PDS gene (PDS21)-upper strand
 <400> 26
 tcgacttaac ttgtaggaa tattaaggag gtaatattcc ttacaagtta ag 52

<210> 27
 <211> 52
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PDS gene (PDS21)-lower strand
 <400> 27
 tcgacttaac ttgtaggaa tattacctcc ttaatattcc ttacaagtta ag 52

<210> 28

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<211> 115
<212> DNA
<213> Artificial sequence

<220>
<223> small hairpin RNA coding region (GUS_A)

<220>
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<222> (1)..(11)
<223> SalI/PvuI restriction sites

<220>
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<222> (12)..(53)
<223> sense RNA encoding region

<220>
<221> misc_feature
<222> (54)..(62)
<223> loop structure

<220>
<221> misc_feature
<222> (63)..(104)
<223> antisense RNA encoding region

<220>
<221> misc_feature
<222> (105)..(115)
<223> SalI/PvuI restriction sites

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gtcgacgatc gtgcggtcac tcattacggc aaagtgtggg tcaataatca ggagttcctt      60
cttcctgatt attgaccac accttgccgt aatgagtgac cgcagtcgac gatcg      115

<210> 29
<211> 112
<212> DNA
<213> Artificial sequence

<220>
<223> small hairpin RNA coding region (GUS_B)

<220>
<221> misc_feature
<222> (1)..(8)
<223> SalI/PvuI restriction sites

<220>
<221> misc_feature
<222> (9)..(50)
<223> sense RNA encoding sequence

<220>
<221> misc_feature
<222> (51)..(59)
<223> loop structure

<220>
<221> misc_feature
<222> (60)..(101)
<223> antisense RNA coding region

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<220>
<221> misc_feature
<222> (102)..(112)
<223> SalI/PvuI restriction site

<400> 29
gtcgacgatc gtcatgaaga tgcggacttg cgtggcaaag gattcgataa gttccttctt      60
tatcgaatcc tttgccacgc aagtccgcac tttcatgacg agtcgacgat cg                  112

<210> 30
<211> 115
<212> DNA
<213> Artificial sequence

<220>
<223> small hairpin RNA coding region (GUS_C)

<220>
<221> misc_feature
<222> (1)..(11)
<223> SalI/PvuI restriction sites

<220>
<221> misc_feature
<222> (12)..(53)
<223> sense RNA coding region

<220>
<221> misc_feature
<222> (54)..(62)
<223> loop structure

<220>
<221> misc_feature
<222> (63)..(104)
<223> antisense RNA encoding region

<220>
<221> misc_feature
<222> (105)..(115)
<223> SalI/PvuI restriction sites

<400> 30
gtcgacgatc gtgcgacctc gcaaggcata ttgcgcgttg gcggtaacaa gaagttcctt      60
ctttcttgtt accgccaacg cgcaatatgc cttgcgaggt cgcagtcgac gatcg                  115

<210> 31
<211> 115
<212> DNA
<213> Artificial sequence

<220>
<223> small hairpin RNA coding region (EIN_A)

<220>
<221> misc_feature
<222> (1)..(11)
<223> SalI/PvuI restriction sites

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